

## ANTIPROLIFERATIVE STUDY OF *BRUCEA JAVANICA* EXTRACTS AGAINST HEAD AND NECK CANCER CELLS

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**Abstract:** *Brucea javanica* is a very useful plant for treatment of many diseases. In order to study the potential use of this plant, crude extracts of the leaves and fruits were assessed for antiproliferative activities against head and neck cancer cell line which is HTB-43. The dried and ground leaves and fruits of the plant were successively extracted using hexane, chloroform, methanol and water, respectively. The crude extracts were subjected to toxicity test using brine shrimp lethality assay. The chloroform extracts from fruits and leaves and methanol extract from fruits induced toxicity against brine shrimps with LC<sub>50</sub> values below 1000 µg/ml. These results indicated that bioactive components presence in the crude extracts for its pharmacologic effects against head and neck cancer cells. Inhibition of growth of the cultured cancer cells line was measured using a standard Microculture Tetrazolium Technique (MTT) assay. Most of the tested crude extracts exhibited significant antiproliferative activities against the HTB-43 cell with IC<sub>50</sub> ranging from 8.46 µg/ml to 47.25 µg/ml. The chloroform extract from the leaves gave the highest antiproliferative activity (IC<sub>50</sub>, 8.46 µg/ml). Methanolic extract of *Brucea javanica* fruit was selected as the most effective extract to inhibit the growth of head and neck cancer cells (HTB-43) by the two different assays used.

**Keywords:** *Brucea javanica*, Meladapahit, HTB-43 Cancer Cell Line, Head and Neck Cancer Cell and Antiproliferative Study

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## Introduction

Head and neck cancer is a squamous cell carcinoma cancer type and most common form of skin cancer. It can be categorized by the area of origin of the head or neck involving upper aerodigestive tract (UADT). There are six overall sites of head and neck region: nasal cavity, pharynx, oral cavity, oropharynx, larynx and hypopharynx (Shane & Woo, 2012). This type of cancer also consists of heterogenous groups of tumors with a multitude of histologies (Lee et al., 2011). The number of head and neck cancer cases is increasing every day, due predominately to the ageing of the populations and the uncontrolled population growth. It is about 500, 000 new cases of the disease reported every year (Lee et al., 2011). Patients in the age group of 60-69 years were the largest percentage of patients with the cancer (Shashinder et al., 2008). Besides, men showed predominance compared with women in the disease (Shashinder et al., 2008). According Scottish Intercollegiate Guidelines Network (SIGN) (2006), there is evidence now days the cancer incidence is increasing amongst young people of both sexes. Smoking and alcohol consumption become well known risk factors for the head and neck cancer (Väisänen et al., 2014). People who are very interested to leave their cigarette on the lip are vulnerable to have lip cancer irrespective of cumulative tobacco consumption. On the other hand, alcohol consumption increases the risk of developing cancers of oral cavity, pharynx and larynx (SIGN, 2006). There is a strong relationship between the quantity of alcohol consumption and the level of risk. Fanucchi et al. (2006) stated that early diagnosis and treatment are important to increase the survival rate of the cancer patients. Besides, any delay may lead to more severe disease, difficult to treat, leading to higher morbidity and mortality (Kowalski & Carvalho, 2001).

In Malaysia, there are several treatments for head and neck cancer conducted by clinical specialist such as surgery, chemotherapy, radiotherapy and palliative care. Those treatments depend on the stage of the cancer had by a patient. According Kahairi et al. (2014), most of the patients with head and neck cancer were being treated by radiotherapy and reconstructive surgery. The delicate nature of the tissues of the UADT is difficult to replace or reconstruct once damage by the disease or the treatment (Shane & Woo, 2012). Chemotherapy and other chemically derived drugs can put patients under a lot of strain and further suffocating their health (Greenwell and Rahman, 2015). The drugs have been developed and undergone trials as well. However, it is difficult to formulate a chemically derived drug which is non-toxic to normal cell and is specific to cytotoxicity of cancer cells (Sameer et al., 2016). Therefore, numerous studies of medicinal plants have been carried out in order to discover new biologically active compounds to reduce the risk of side effects from chemically derived drugs treatment.

Cancer amongst the human population share similar characteristics or genotypes such as insensitivity to signals which inhibit cell growth their unlimited replication (Greenwell and Rahman, 2015). Besides, apoptosis incidence is evaded and never induced in cancer cells and angiogenesis is sustained within the tumor tissue allowing survival of cancer cells (Greenwell and Rahman, 2015). Plant derived compounds in plant have demonstrated properties to inhibit proliferation of cancer cells and inducing apoptotic cells death. The compounds which have been identified for their antiproliferative properties include polyphenols, brassinosteroids, taxols and flavonoids.

*Brucea javanica* or locally known as Meladapahit, is being used in Malaysia as traditional medicine mainly for the treatment of diabetes mellitus and hypertension. It is a member of Simaroubaceae family. It is a shrub tree with 1 to 3 meters and younger parts softly

pubescent. It also has compound-paripinnate leaves and the flowers are minute, purple, in numerous small cymes or clusters collected into axillary panicles. The fruit becomes black when ripened. Recent studies found this plant has potential for the treatment of inflammatory diseases and induced cytotoxicity and apoptosis in many cancer cell lines. Lau et al. (2005) have demonstrated aqueous extract of *B. javanica* on four human carcinoma cancer cell lines and found that the extract possessed antiproliferative and apoptosis inducing properties. Researchers have now isolated several natural compounds from *B. javanica*. Tetracyclic tetrapene quassinoids are the main active components of *B. javanica* with remarkable antitumor activity (Chen et al., 2013). Recent research found that the quassinoids have acted effectively in the treatment of several diseases such as leukemia and exert significant anti-inflammatory activity (Chen et al., 2013). But, it has been done only with single fraction (usually methanol or aqueous) instead of trials by using different solvents for comparison purposes. Besides, the use of a single solvent does not ensure the extraction of all the phytochemicals present in a plant. Therefore, the present study was carried out to demonstrate the *in vitro* antiproliferative of sequentially extracted different solvent extracts of fruits and leaves of *B. javanica* against a cancer cell line. Sequential extraction is the method extracting plant using different solvents in the increasing polarity order (Jeyaseelan et al., 2012). This study is important to evaluate the cytotoxicity of the plant extracts against the selected head and neck cancer cell line which is HTB-43 (pharynx cancer cells).

## **Methodology**

### ***Plant Materials***

*B. javanica* fruits and leaves were collected from a local farmer at Gemencheh, Negeri Sembilan, Malaysia. The collected specimens were thoroughly washed under running tap water and then oven-dried at 60°C for three days. The dried fruits and leaves of *B. javanica* were ground to a coarse powder using grinding machine.

### ***Preparation of B. Javanica Extracts***

The powdered fruits and leaves of *B. javanica* (100 g each) were subjected to sequential solvent extraction with hexane, chloroform, methanol and water according to Pathmanathan et al. (2010). This process involved maceration with each solvent for two cycles; each cycle involving one day soaking and shaking using wrist shaker at room temperature. The next solvent was added to the residue after filtration and the extraction was carried out for the next 2 days in a similar way. The extracts were filtered and concentrated using a rotary evaporator (Buchii, Switzerland) under reduced pressure at 40°C to yield a concentrated hexane, chloroform and methanol extracts. The extracts were preserved in vacuum oven for further use. Hot aqueous extracts of fruits and leaves of *B. javanica* were prepared by further boiling the residue from the previous filtration step in ultra-pure water for 3 hours. The hot aqueous extracts were filtered using Whatman filter paper No. 54 and spray dried to give the powdered form.

### ***General Cell Culture Methods***

Chemicals and reagents used in the cell culture experiments are Gibco products that purchased from Bio-Diagnostics, Malaysia. The human pharynx cancer cell line used in the study, HTB-43 was purchased from American Type Culture Collection (ATCC). For general cell culture, the HTB-43 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented

with 10% Fetal Calf Serum (FCS), Glutamax (100X) and Penicillin and Streptomycin (100X) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C.

### ***Brine Shrimp Lethality Assay***

Brine shrimp (*Artemia salina* Leach) dried eggs were hatched in a shallow two compartment rectangular plastic box filled with artificial sea water (36 g/L) which was prepared from commercial sea salt (Sigma Chemical Co., UK) and sterilized distilled water. A divider with several holes was placed in between the covered and the open compartment. The eggs were placed into the dark section, while the open compartment was illuminated. After 48 hours of incubation at room temperature (30°C), nauplii (larvae) were collected from the lighted side whereas their shells and other unhatched eggs were left in the light tight side.

The brine shrimp lethality test was conducted by using the 96-well microplates procedure described by Solis et al. (1993). An aliquot (100 µl) of the 2 mg/ml sample solution was dispensed in triplicate into the first and second well of the microplate row. Two fold serial dilutions with 100 µl sea salt solution were made in triplicate across the plates starting from well number 2 to 8 to give final concentration of 7.8 µg/ml. 2% of DMSO diluted with sea salt water was used as a solvent and also as a negative control. 7 – 10 mature nauplii in suspension were then added into each well and the covered plates were incubated in room temperature for 24 hours. The numbers of survivors were counted and LC<sub>50</sub> values (lethality concentration by 50%) were analyzed after the incubation. The LC<sub>50</sub> values were determined using the probit analysis by IBM SPSS 20.

### ***Antiproliferative Activity Assay***

Inhibition of growth of the cultured cancer cells line was measured using a standard MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. The assay detects the reduction of yellow MTT dye by metabolically active cells, in part by action of mitochondrial dehydrogenase of viable cells to purple formazan crystals (Wiratchanee et al., 2010).

The cells were seeded in 96-well flat bottom plates at a density of 105 cells/well in 100 µl culture medium and allowed to attach during 24 hours incubation time. The cells were then treated with different concentration of plant extracts (100 µl each well) and incubated for 72 hours. There were eight concentrations prepared from each extract (500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.9 µg/ml). The extracts that derived from non-polar solvents were dissolved in dimethylsulfoxide (DMSO) and the aqueous extract was dissolved in distilled water before the treatment. Untreated cells were used as a negative control in the study.

Next, 20 µl of 5 mg/ml of MTT reagent was added into each well, and the plates were incubated for 4 hours at 37°C. After the incubation, the remaining MTT solution removed and 100 µl DMSO was added into each well of the plates to dissolve the purple formazan and lysed the cell to release the mitochondrial residues of formazan. Absorbance measurements were made at 570 nm (Ong et al., 2017) and IC<sub>50</sub> values (concentration that inhibit cell proliferation by 50%) were obtained by using EnSpire Multimode Plate Reader. All the experiments were performed in triplicate and repeated three times in order to perform the statistical analysis.

## Results

### *Percentage Yield of Extracts*

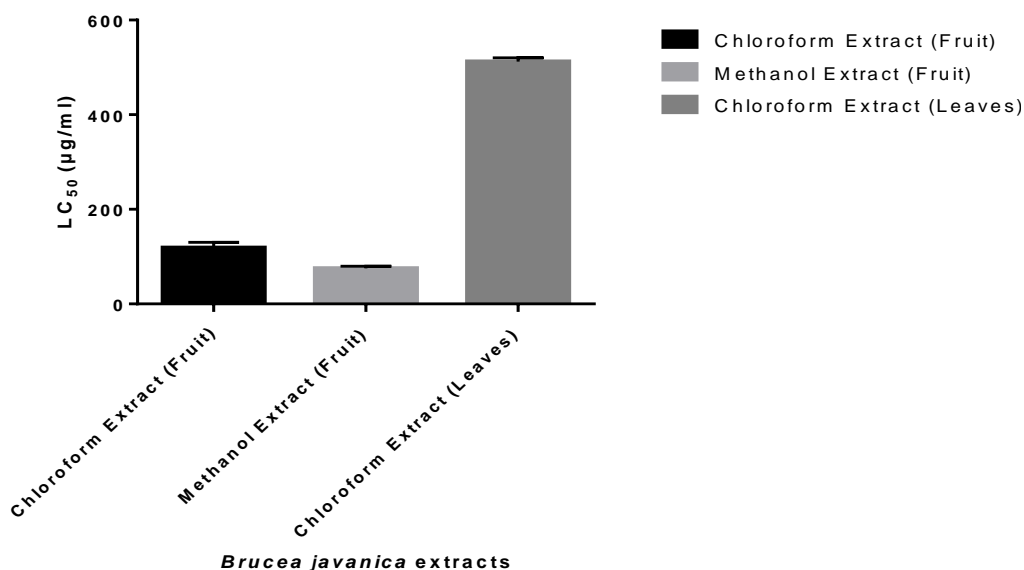
Table 1 revealed the results of weight of solute extracted from 200 g of powdered fruits and 131 g of powdered leaves. Besides, the percentage yield of the plant crude extracts using different solvents also presented in the table. The results showed that aqueous extract of the fruits produced the highest yield with 16.36 g representing 8.18 % followed by hexane extract with 10.38 g having 5.19 % and the least was the chloroform extract with 1.05 g representing 0.53 %. On the other hand, for leaves extracts, methanol extract produced the highest yield with 15.95 g representing 12.18% and the least was the chloroform extract with 4.74 g representing 3.62 %.

**Table 1: Percent Yield of Aqueous and Organic Solvent Extracts of *B. Javanica* Fruit and Leaves**

Extract	Fruit			Leaves		
	Weight of the powdered sample (g)	Weights of sample extract (g)	Percent (%) yield	Weight of the powdered sample (g)	Weights of sample extract (g)	Percent (%) yield
Hexane	200	10.38	5.19	131	5.17	3.95
Chloroform	200	1.05	0.53	131	4.74	3.62
Methanol	200	4.39	2.2	131	15.95	12.18
Aqueous	200	16.36	8.18	131	4.9	3.74

### *LC<sub>50</sub> Values of Brine Shrimp Lethality Assay*

The brine shrimp lethality test results are presented in Figure 1. Out of eight extracts screened for activity against brine shrimp larvae, three of the crude extracts demonstrated activity below 1000 µg/ml.



**Figure 1: Brine Shrimp Lethality Assay Results of *Brucea Javanica* Crude Extracts. Only Three Extracts Induced Toxicity against The Nauplii. Data Represents Mean ± SD Values in Three Replicates.**

There were chloroform extracts from fruits and leaves and methanol extract from fruits with LC<sub>50</sub> values of 118.7±11.32 µg/ml, 512.44±7.9 µg/ml and 75.27±4.33 µg/ml respectively (Table 2). Other extracts were considered to be non-toxic towards the *Artemia salina* larvae (LC<sub>50</sub> values >1000 µg/ml).

**Table 2: The Mean LC<sub>50</sub> Values of the Active Crude Extracts by Brine Shrimp Lethality Assay.**

LC <sub>50</sub> value (µg/ml)	Plant Parts	
	Fruits	Leaves
Chloroform	118.7±11.32	512.44±7.9
Methanol	75.27±4.33	>1000

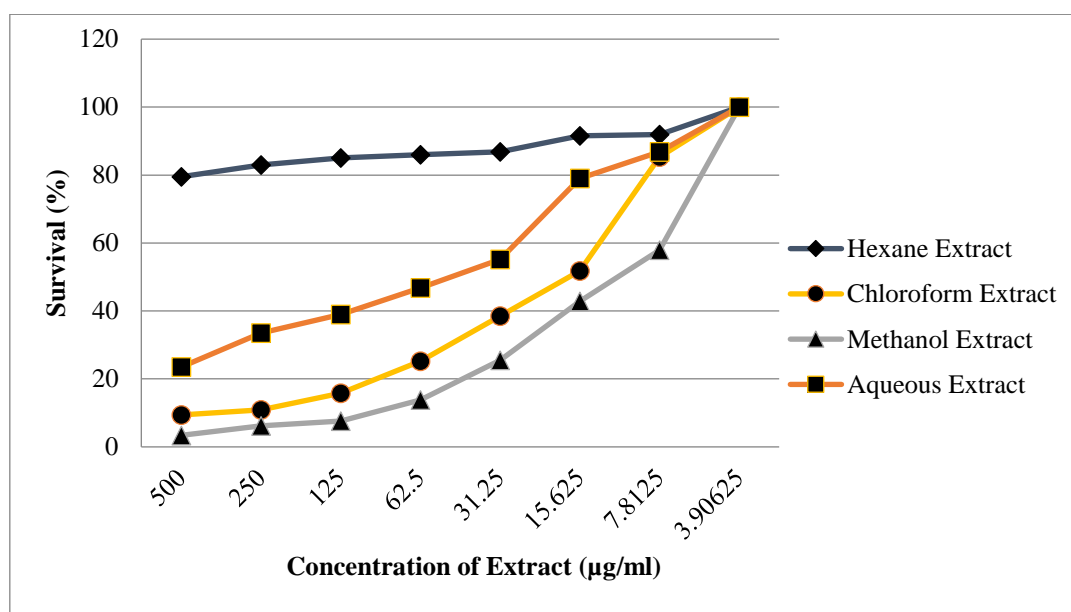
### *Antiproliferative Activity of *Brucea Javanica* Extracts*

Most of the *Brucea javanica* crude extracts exhibited antiproliferative activities against the HTB-43 cells as showed in Table 3.

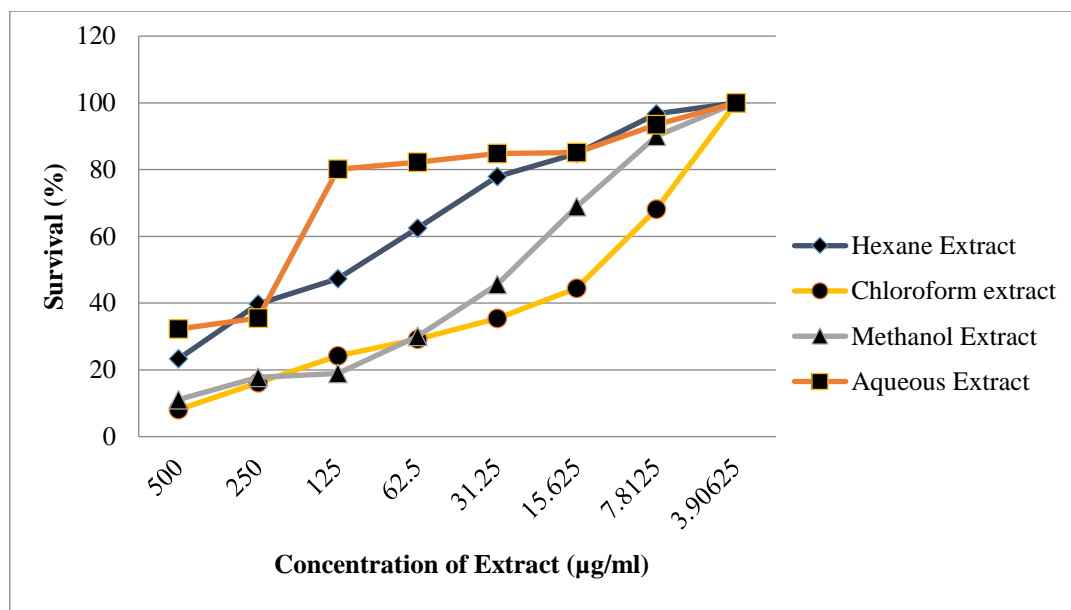
**Table 3: IC<sub>50</sub> Values of the *Brucea Javanica* Extracts against Human Pharynx Cancer Cell Line (HTB-43) By MTT Assay.**

IC <sub>50</sub> value (µg/ml)		
Fraction	Plant Parts	
	Fruits	Leaves
Hexane	>500	103.95±10.38
Chloroform	15.86±4.54	8.46±2.3
Methanol	8.52±1.61	26.48±4.42
Aqueous	47.25±8.24	195.68±6.99

The percentage survival of head and neck cancer cells (HTB-43) versus the concentration of *B. javanica* extracts are shown on Figure 2 and Figure 3.

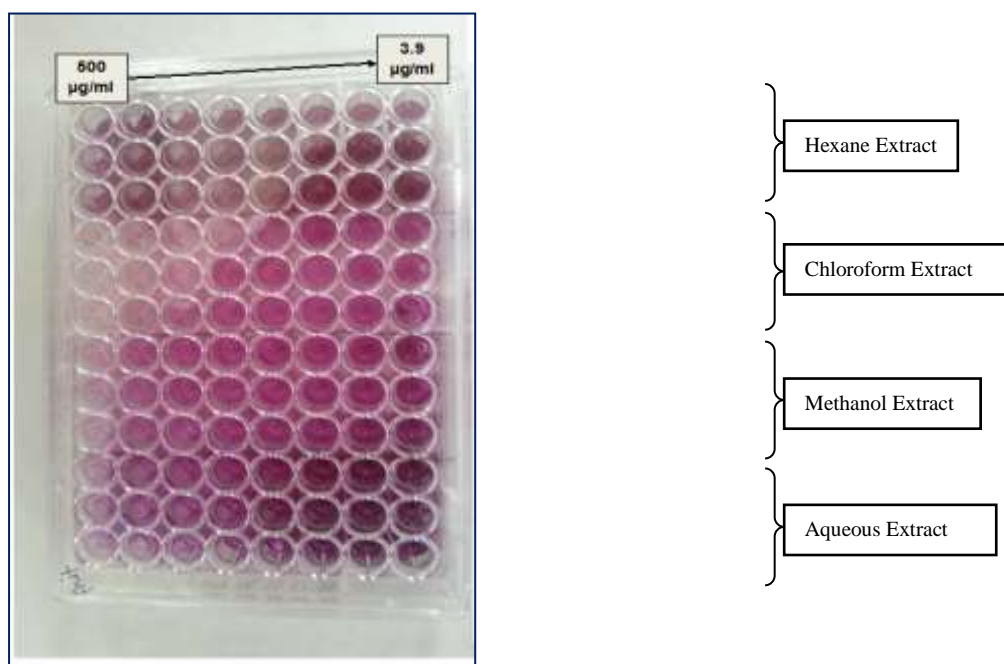


**Figure 2: The Percentage Survival of Cells (HTB-43) Versus the Concentration of *B. Javanica* Fruits Extracts.**



**Figure 3: The Percentage Survival of Cells (HTB-43) Versus the Concentration of *B. Javanica* Leaves Extracts.**

The survival rate of the cells is increasing when treated with the lowest concentration of the extracts. The reduction of yellow MTT dye to purple formazan crystals described the antiproliferative activities of the tested extracts (Figure 4).

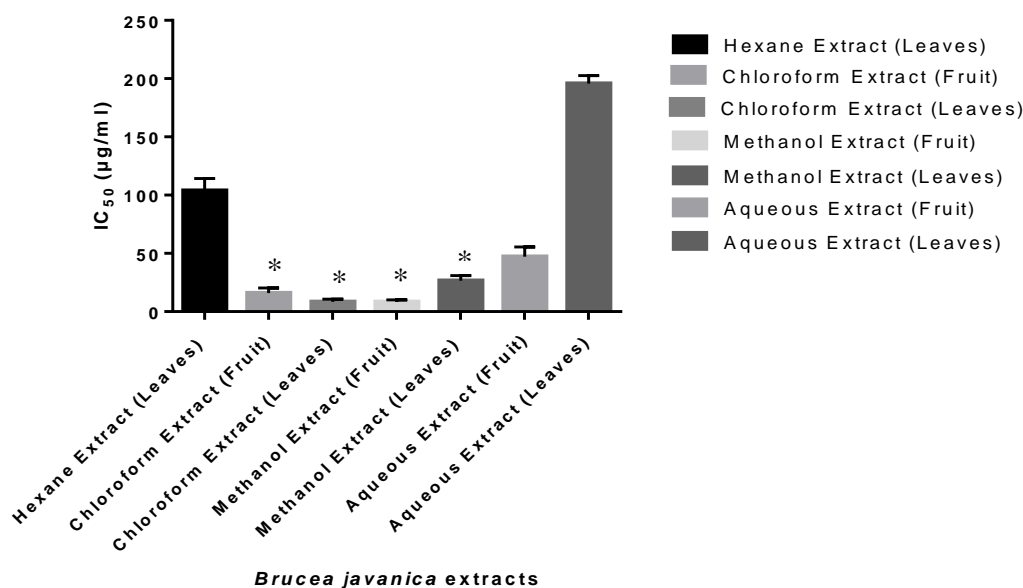


**Figure 4: The Reduction of Yellow MTT Dye to Purple Formazan Crystals from 500 µg/ml of 3.9 µg/ml of Extracts Treatment.**

From the results, it can be suggested that chloroform extract (leaves) of the plant expressed the highest inhibition towards the HTB-43 cell line with  $IC_{50}$  value of  $8.46 \pm 2.3$  µg/ml. Other potential extracts are chloroform extract of the fruit and methanol extract of the fruit and leaves with  $IC_{50}$  values of  $15.86 \pm 4.54$  µg/ml,  $8.52 \pm 1.61$  µg/ml and  $26.48 \pm 4.42$  µg/ml



respectively (Table 3 and Figure 5). Hexane extract from the fruits, hexane and aqueous extracts from *B. javanica* leaves showed low antiproliferative activities to the HTB-43 cell line with an IC<sub>50</sub> values >100 µg/ml.



**Figure 5: There were Four Potential Extracts (Chloroform and Methanol Extracts from Plant Parts, Fruit and Leaves) with Antiproliferative Activities against the Cancer Cell Line. Data Represents Mean ± SD Values in Three Replicates.\*The Potent Extract**

## Discussion

The extraction yield of a plant is depends on factors such as choice of solvent, solvent concentration, the solvent to solid ratio, extraction period, extraction temperature and particle size of the plant material (Silva et al., 2007).For *B. javanica* fruits extraction, aqueous solvent extracted the highest yield because of water contains higher polarity compared with other solvents and most of the compounds extracted are hydrophobic.Leaves extraction of the *B. javanicashows* that methanol solvent extracted the highest yield of the plant material because of mostly methanol is used for extraction various polar compounds but certain groups of non-polar compounds are fairly soluble in methanol.

Brine shrimp lethality assay is one of the best and rapid tests for biological and toxicological purposes in a lab (Kanwar, 2007). An extract is considered active when the LC<sub>50</sub> values lower than 1000 µg/ml (Khade et al., 2011). In addition, Rieser et al. (1996) reported that crude extract resulting in LC<sub>50</sub> value less than 250 µg/ml were considered significantly active and had potential for further investigation. Based on the results, the chloroform and methanol extracts of *B.javanica* fruit have the potential to be the candidate for the investigation of cytotoxic compounds due to the LC<sub>50</sub> values obtained were <250 µg/ml. Chloroform extract from the plant leaves was found to be less toxic in the study. The result was supported by Marissa et al. (2012) where they categorized the *B.javanica* Merrill leaves extract was slightly toxic on mice.

MTT assay is an adequate method to study the cell viability and proliferative activities on cell (Imbert & Cullander, 1999). The effect of extracts on the growth of cells in vitro was estimated by the reduction of the yellow MTT dye. According to American National Cancer

Institute by Itharat et al. (2004), to meet the criteria of cytotoxic activity, any crude extracts need to have an  $IC_{50} < 30 \mu\text{g/ml}$ . In the study, the chloroform and methanol extracts of both plant parts (fruit and leaves) were found to significantly reduced HTB-43 cells proliferation. A study by Li et al. (2008) found that methanol extract of *B.javanica* of the combined twigs, leaves and inflorescence showed high antiproliferative activity against MCF-7 human breast cancer cell line. Besides, *B.javanica* fruit extract by ethanol solvent induced cytotoxicity and apoptosis in pancreatic adenocarcinoma cancer cell line (Sin et al., 2008). The studies were supported the results in this research, where the methanolic extract of *B.javanica* exhibit potent cytotoxic activity against tested cell line. Chloroform extract has never been used to isolate bioactive compound in previous research. The data on **Table 1** indicated that the chloroform inhibited the cell growth with the highest  $IC_{50}$  value compared with others. It seems that the used of chloroform for extraction is strongly useful.

## Conclusion

From the study, brine shrimp lethality assay and MTT assay were used for evaluation of cytotoxic activity by *B.javanica* fruit and leaves extracts. Based on the results and discussion above, we concluded that chloroform and methanolic extracts of the plant fruit exhibit potent antiproliferative property against the head and neck cancer cell line (HTB-43). However, methanolic extract of *B.javanica* fruit was selected as the most effective extract to inhibit the growth of HTB-43 cells due to having the lowest  $IC_{50}$  and  $LC_{50}$  values. This extract should be tried on normal cells in order to measure the potential difference in cytotoxicity which could make these findings more valuable. Further research on phytochemical evaluation and isolation of active compounds from methanolic extract of *B. javanica* fruit are essential for the development of new antiproliferative agents against head and neck cancer. Furthermore, drug delivery studies are required to know the bioavailability of the agents *in vivo*.

## References

- Chen, M., Chen, R., Wang, S., Tan, W., Hu, Y., Peng, X. & Wang, Y. (2013). Chemical components, pharmacological properties, and nanoparticulate delivery systems of *Brucea javanica*. *International Journal of Nanomedicine*, 3(8), 85 – 92.
- Fanucchi, M., Khuri, F.R, Shin, D., Johnstone & P.A.S, Chen, A. (2006). Update in the management of head and neck cancer. *Update on Cancer Therapeutics*, 1, 9-211.
- Greenwell, M. & Rahman, P. K. S. M. (2015). Medicinal plants: Their use in anticancer treatment. *International Journal of Pharmaceutical Science and Research*, 6(10), 4103 – 4112.
- Imbert, D. & Cullander, C. (1999). Buccal mucosa in vitro experiments I. Confocal imaging of vital staining and MTT assays for the determination of tissue viability. *Journal of Controlled Release*, 58, 39-50.
- Itharat, A., Houghton, P.J, Eno, A.E, Burke, P.J, Sampson, J.H. & Raman, A. (2004). In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *Journal of Ethnopharmacology*, 90, 33-38.
- Jayaseelan, E. C., Jenothiny, S., Pathmanathan, M. K. & Jayadevan, J. P. (2012). Antibacterial activity of sequentially extracted organic solvent extracts offruits, flowers and leaves of *Lawsonia inermis* L. from Jaffna. *Asian Pacific Journal of Tropical Biomedicine*, 2(10), 798 – 802.
- Kahairi, A., Raja, A.R.L.A, Zamzil, A.A, Mohd, S.R. & Wan, I.L. (2014). An outcome of surgically treated head and neck cancer in one of the tertiary referral center in the east

- coast of Malaysia: A 6-year retrospective analysis. *Malaysian Journal of Medical Science*, 21(4), 28-36.
- Kanwar, A.S. (2007). Brine shrimp (*Artemia salina*) a marine animal for simple and rapid biological assays. *Journal of Chinese Clinical Medicine*, 2, 236-240.
- Khade, K.V., Dubey, H., Tenpe, C.R., Yeole, P.G. & Patole, A.M. (2011). Anticancer activity of the ethanolic extracts of *Agave americana* leaves. *Pharmacologyonline*, 2, 53-68.
- Kowalski, L.P & Carvalho, A.L. (2001). Influence of time delay and clinical upstaging in the prognosis of head and neck cancer. *Oral Oncology*, 37(1), 8-94.
- Lau, F. Y., Chui, C. H., Gambari, R., Kok, S. H., Kan, K. L., Cheng, G. Y., Wong, R. S., Teo, I. T, Cheng, C. H., Wan, T. S., Chan, A. S. & Tang, J. C. (2005). Antiproliferative and apoptosis-inducing activity of *Brucea javanica* extract on human carcinoma cells. *International Journal of Molecular Medicine*, 16(6), 1157 – 1162.
- Lee, S.C, Tang, I.P, Avatar, S.P, Ahmad, N., Selva, K.S, Tay, K.K, Vikneswaran, T. & Tan, T.Y. (2011). Head and neck cancer: Possible causes for delay in diagnosis and treatment. *Medical Journal of Malaysia*, 66(2), 101-104.
- Li, P., Chin, Y.W., Chai, H.B., Ninh, T.N., Soejarto, D.D. & Kinghorn, A.D. (2008). Bioactivity-guided isolation of cytotoxic constituents of *Brucea javanica* collected in Vietnam. *Bioorganic and Medicinal Chemistry*, 17(2009), 2219-2224.
- Marissa, A., Indah, D.D, Banjarnahor, S.D.S., Megawati & Tri, Y. (2012). Acute toxicity of *Brucea javanica* Merrill leaves extract on mice. *The Journal of Tropical Life Science*, 2(2), 29-31.
- Ong, T. H. D., Yu, N., Meenashisundaram, G. K., Schaller, B. & Gupta, M. (2017). Insight into cytotoxicity of Mg nanocomposites using MTT assay technique. *Material Science and Engineering: C*, 78, 647 – 652.
- Pathmanathan, M.K, Uthayarasa, K., Jeyadevan J.P. & Jeyaseelan E.C. (2010). *In Vitro* antibacterial activity and phytochemical analysis of some selected medicinal plants. *International Journal of Pharmaceutical & Biological*, 1(3), 291-299.
- Rieser, M. J., Gu, Z.-M., Fang, X.-P., Zeng, L., Wood, K. V., & McLaughlin, J. L. (1996). Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*. *Journal of Natural Products*, 59, 100-108.
- Sameer, R., Nidhi, S., Tarun, V., Charan, S. & Jyoti, G. (2016). A review on naturally derived compounds for potential anticancer activity. *Indian Journal of Drugs*, 4(3), 75 – 86.
- Scottish Intercollegiate Guidelines Network (SIGN). (2006). Diagnosis and management of head and neck cancer. *A national Clinical Guideline, Edinburgh*, pp.1-78.
- Shane, L. & Woo, Y.Y. (2012). Principles and management of head and neck cancer. *Elsevier Journal*, 30(11), 617-623.
- Shashinder, S., Choo, P.K, & Gopala, K.G. (2008). Outcome of patients with head and neck cancers: 10-year experience of an otorhinolaryngology – head and neck unit in a tertiary hospital of a developing country. *European Journal of Cancer Care*, 17, 93–97.
- Silva, E. M., Rogez, H., & Larondelle, Y. (2007). Optimization of extraction of phenolics from *Inga edulis* leaves using response surfacemethodology. *Separation and Purification Technology*, 55(3), 381–387.
- Sin, T.L., Lin, Z.X., Ming, Z. & Po, S.L. (2008). *Brucea javanica* fruit induces cytotoxicity and apoptosis in pancreatic adenocarcinoma cell lines. *Phytotherapy Research*, 22, 477 – 486.
- Solis, P. N., Wright, C. W., Anderson, M. M., Gupta, M. P., & Phillipson, J. D. (1993). A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Medica*, 59, 250-252.

- Väisänen, J.A., Syrjälä, A.M., Pesonen, P.R., Pukkila, M.J., Koivunen, P.T. & Alho O.P. (2014). Characteristics and medical-care-seeking of head and neck cancer patients: A population-based cross-sectional survey. *Oral Oncology*, 50, 740-745.
- Wiratchanee, M., Vithoon, V., Wanna, C., Arunporn, I. & Kesara, N.B. (2010). Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells in vitro. *BMC Complementary and Alternative Medicine*, 10(55), 1-8.